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EXCRETION AND METABOLISM OF d-AMPHETAMINE SULFATE IN THE CAT

BY

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The undersigned certify that they have read and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Excretion and Metabolism of d-Amphetamine Sulfate in the Cat", submitted by Douglas A.J. Levy in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

The objective of this investigation was to study the metabolism and excretion of d-amphetamine in the cat. ^{14}C-amphetamine sulfate was selected for this purpose. Five male cats of approximately the same age and weight were used for this experiment.

Blood samples were taken from the animals at 15 min, 30 min, 1 hr, 6 hrs, 12 hrs, and 24 hrs after administration of the labeled drug. From the data obtained it is difficult to determine the time at which the blood level was the highest. It would appear from this experiment, however, to occur at 1 hr following intraperitoneal injection.

Urine samples were taken every 24 hrs for four days. It was observed that the maximum urine level was obtained 48 hrs after the intraperitoneal injection of the drug.

Thin layer chromatography combined with autoradiography was the method of choice used in the separation, and identification of the urinary metabolites. The qualitative study of the autoradiograms demonstrated the possibility of the urine samples containing at least seven metabolites. Four of the seven possible metabolites were identified as: unchanged amphetamine sulfate, p-hydroxyamphetamine, hippuric acid, and nor-ephedrine. Benzoic acid was not found to be a metabolite.

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I. INTRODUCTION

Drug metabolism may be defined as all the changes involved in the utilization of a particular drug by the body. Most compounds are metabolized before being excreted in the urine, bile, or expired air. Therefore, the duration of drug action is generally limited by the rate of conversion of therapeutic agents to active, and inactive metabolites. Usually the original drug, with very few exceptions, is at least partially transformed into metabolites having new physical and chemical properties. Codeine and ephedrine, for example, are converted to metabolites having pharmacologic properties similar to those of the parent drug. Others, like imipramine produce their therapeutic effects by the formation of pharmacologically active metabolites.

It has been shown from numerous studies that the majority of drugs are metabolized by surprisingly few reactions. The major reactions are: oxidation, reduction, hydrolysis and conjugation synthesis. The enzyme systems that catalyze these metabolic alterations are present mainly in the microsomes which are derived from the endoplasmic reticulum of the liver cells. The endoplasmic reticulum consists of lipoidal tubules that extend in all regions of the cytoplasm. Upon homogenization, this network of tubules is broken and forms small vesicles of which two types can be isolated. Those portions of endoplasmic reticulum which have no granular material are called "smooth" and those that contain ribosomes are called "rough". It is the "smooth" microsomal portion that is mainly responsible for the metabolism of drugs.

Amphetamine which is racemic β-phenylisopropylamine is

one of the most import "sympathomimetic" drugs used in medicine today. It is used mainly as a CNS stimulant for the treatment of narcolepsy, alcoholism, mental depression, postencephalitic parkinsonism, barbiturate or morphine poisoning and fatigue. It is also used for spasms of the gastrointestinal tract, dysmenorrhea, hypotension and to produce anorexia in obesity.

It was the purpose of this investigation to study the urinary excretion and metabolism of d-amphetamine in the cat.

II. SURVEY OF LITERATURE

A. Chemistry of Amphetamine.

Amphetamine sulfate (racemic β-phenylisopropylamine sulfate) is a white, odorless, crystalline powder having a molecular weight of 368.49, and a melting point above 300°C with decomposition. The compound is soluble one part in 8.8 parts of water and 3 parts of boiling water. It is insoluble in ether and only one part dissolves in 515 parts of 95% alcohol. A solution of one gram in 10 cc of water has a pH of between 5.0 and 6.0. The base is separated by the addition of sodium hydroxide to the aqueous solution. The free base is a colorless oily liquid with a sharp characteristic amine odor. It is readily soluble in acids, soluble in alcohol, ether, benzene, acetone and is slightly soluble in water. The boiling point is 200 - 203°C at atmospheric pressure and 82 - 85°C under 13 mm mercury pressure (1). The amine, when treated with sodium hydroxide and benzoyl chloride, yields an amide which when crystallized from dilute alcohol melts at 134 - 135°C. Amphetamine produces a stable purple color when treated with hydroxylamine. Such a color could be used for its colorimetric determination (2).

The methods of synthesis of amphetamine and in general the benzyl-alkyl amines of the form C_6H_5-CH_2-CH X -NH_2, where X may be an alkyl or aryl group, consist of the reduction of the corresponding benzyl-alkyl-ketoximes. The hydrogenation of the latter may be done by electrolysis in a solution of sulfuric acid at a particular temperature (3). Methylcinnamic acid may also serve as starting material by hydrogenation and the subsequent degradation of the amide by the Hofmann reaction (4).

The reaction of Leukart consisting of the action of formamide, ammonium formate and formic acid on phenyl acetone, then followed by the hydrolysis of the intermediary formyl derivate, yields amphetamine up to 70%. The latter was separated by alkalinization and steam distillation (1, 5).

The synthesis of amphetamine starting from O-Chlorophenyl acetic acid whose acid chloride is treated with methyl iodide in the presence of zinc is of theoretical interest. The product of the reaction is hydrolyzed to give O-Chlorophenyl acetone. The treatment of the ketone with formamide under reflux yields the formyl derivative of the corresponding amine which is hydrolyzed either in alkaline medium or with hydrochloric acid to O-Chlorobenzyl methylamine. The last product is first hydrogenated in the presence of palladium in alcohol-acetic acid solution, then again dehydrogenated to yield amphetamine (3).

B. Pharmacology of Sympathomimetic Amines

Oliver and Schafer (6), in a paper published in 1895, mentioned that Pellacani, working alone in 1874 and in conjuction with Foa in 1884, appears to have been the first to investigate the effect of injecting extracts of suprarenal capsules into animals.

In this paper, Oliver and Schafer (6) showed that hypertension could be produced by the injection of adrenal extracts into animals. These workers stated the following:

> "The material which they form and which is found (in the suprarenal capsules) at least in its fully active condition only in the medulla of the gland produces striking physiological effects upon the muscular tissue generally and especially upon that of the heart and arteries".

Abel and Crawford (7) in 1897 partially separated from the adrenal extracts a compound that raised the blood pressure of vertebrates. The complete isolation of this compound, which was epinephrine, was accomplished by Takamine and Aldrich in 1901, as stated in a paper published by Dakin in 1905 (8).

Elliott in 1904 (9) suggested that epinephrine is released from the sympathetic nerve endings. This theory was adopted and developed further by Dixon and Hamill in 1909 (10).

An important study was done in 1910 by Barger and Dale (11) in which they studied the pharmacological activity of a large series of synthetic amines on the blood pressure of decerebrate cats. They found that phenyl-propylamine was pharmacologically related to epinephrine and they termed its action "sympathomimetic".

However, in 1924 Chen (12) separated from the plant Ma huang a substance, ephedrine, whose effects lasted longer than epinephrine. The stability of this separated compound presented the major advantage of its ability to be administered orally.

Amphetamine, on the other hand, was synthesized by Alles (13) in 1924 and its action was studied in relation to other phenylalkyl amines. The latter comprised three important amphetamines, two of which are optical isomers of each other and the third is a methyl

derivative of the racemic mixture. Table I demonstrates the structural differences between the different amphetamines and the naturally occurring substances, epinephrine, nor-epinephrine and ephedrine.

The sympathomimetic drugs comprise one of the most extensively studied groups of pharmacological agents and consequently many are known and widely used.

The actions of most sympathomimetic agents can be classified into five broad types (14):

(1) A peripheral, excitatory action on certain types of smooth muscle, such as those in blood vessels supplying mucous membranes, skin, and also on salivary gland secretion.

(2) A peripheral inhibitory action on certain other types of smooth muscle, such as those in the bronchial tree, in the wall of the gut, and in blood vessels supplying skeletal muscle.

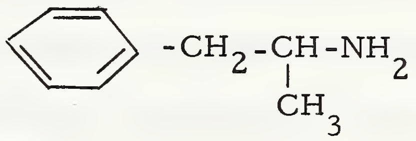
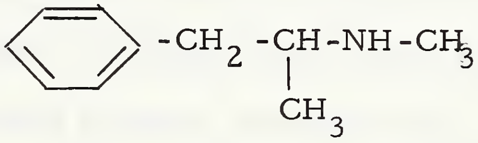
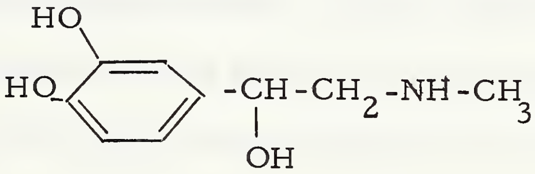
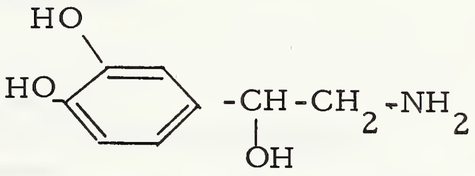
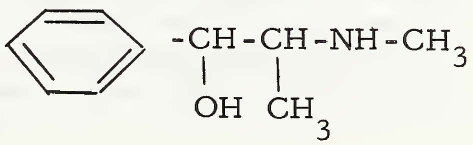
(3) Metabolic actions, such as liberation of free fatty acids from adipose tissue and an increase in rate of glycogenolysis in liver and muscle.

(4) A cardiac excitatory action, responsible for an increase in both heart rate and force of contraction.

(5) CNS excitatory actions such as respiratory stimulation and, with some of the drugs such as amphetamine, a reduction in appetite and an increase in wakefulness.

Table I

Structural differences between the common Amphetamines and the Biogenic Amines.

Name	Structural Formula	Dose
Amphetamine		Racemic: 8-25 mg orally
Methyl Amphetamine		10-30 mg I.M. or I.V. 2.5 to 10 mg orally
Epinephrine		0.5 mg every 8-16 hrs I.M.
Nor-epinephrine		0.5-1.0 mg I.M.
Ephedrine		20-40 mg orally

Amphetamine may be classed as one of the most potent sympathomimetic amines with respect to stimulation of the CNS. The d-isomer (Dextroamphetamine) being three to four times as potent as the l-isomer (15).

The central stimulant effects of amphetamine were first used clinically by Prinzmetal and Bloomberg in 1935 to treat narcolepsy (16). Many experimental and clinical studies of its central excitatory and analeptic properties have been made comparing its potency with such drugs as strychnine, picrotoxin, nikethamide, and caffeine (15).

There is little question that amphetamine is an effective agent for stimulating the medullary respiratory centre, lessening the degree of central depression caused by various drugs, and stimulating the normal cerebrospinal axis. Animals which were given sufficient doses of amphetamine show tremor, restlessness, increased motor activity, agitation and sleeplessness. These effects are thought to be due to cortical stimulation of the reticular-activating system (15).

In man, the main effects of an oral dose of 10-30 mg of d-amphetamine are (15, 17):

(1) Wakefulness
(2) Decreased sense of fatigue
(3) Alertness
(4) Increased motor and speech activity
(5) Elevation of mood with increased initiative, confidence and ability to concentrate, often with elation and euphoria

FATIGUE - Extensive studies have been done in the laboratory concerning the prevention and reversal of fatigue by amphetamine in athletics, and in the military field. Generally, the duration of

adequate performance is prolonged before fatigue appears and the effects of fatigue are partly reversed. The most striking improvement due to amphetamine seems to occur when performance has been reduced by fatigue. It has been suggested that such improvement may possibly be due to alteration of unfavourable attitudes toward the task. It must be remembered, however, that the need for sleep may be postponed, but it cannot be avoided indefinitely (17,18).

MENTAL DEPRESSION - Marked analeptic action in man is exemplified by the fact that anesthesia produced by 0.5 g of amobarbital sodium given intravenously can be abolished by 10 to 30 mg of amphetamine injected intravenously. The psychic effects, however, depend on the dose given, as well as the mental state and personality of the individual (16).

APPETITE DEPRESSION - One of the most common uses of amphetamine today is in the treatment of obesity. The appetite depressant effects of amphetamine were first reported by Nathanson in 1939 (19) and by Harris and others in 1947 (20). Harris reported a loss of weight in dogs and in normal and obese humans treated with amphetamine. The weight loss was almost entirely due to reduced food intake, and only in a small measure to increased metabolism. The precise site of this action of amphetamine has not as yet been determined but is believed to be located in the hypothalamus (21).

Tolerance to the drug, however, develops in man with continued administration (22,24). In obese individuals, the degree of appetite suppression is not enough to reduce weight continuously

without additional dietary restrictions (23).

CONTRAINDICATIONS - abuse of amphetamine as a means of overcoming sleepiness and of increasing energy and alertness should be discouraged. Amphetamine should be used with caution in patients with anorexia, insomnia, asthenia, psychopathic personality, or a history of homicidal or suicidal tendencies (24).

Appendix I gives a list of most of the amphetamine products available on the Canadian market. It can be clearly seen that there are a large number of preparations available in various dosage forms, indicating the popularity of amphetamine.

Bartholomew (25) feels that amphetamines are "too popular" when he states in his paper that:

> "Whether or not the amphetamines are of value in a few conditions the fact remains that the drugs are (i) dangerous in terms of producing dependency; (ii) altogether too freely available, and (iii) far too freely prescribed".

C. Absorption, Excretion and Metabolism Studies

Human studies, conducted by Richter in 1938, showed that 40% of a 20 mg dose of Benzedrine sulfate given orally could be extracted from the urine in 24 hours (26). This value rose to 70% in 48 hours.

It was believed by Beyer, Harris, and others (27,28) that a portion of the drug was catabolized in some fashion by the liver. Several studies were made to determine the pharmacokinetics of amphetamine in man by Chapman, Beckett and others (29,30,31,32).

Dring and others reported in 1966 that amphetamine itself is a major excretory product in man being about 30% of a 0.07 mg/kg dose of ^{14}C-amphetamine sulfate (33). The other metabolites found in the 24 hr urine sample were:

(1)	p-hydroxyamphetamine	3%
(2)	Benzyl-methyl-ketone [(a)]	3%
(3)	Benzoic acid	20%

In a more recent study Dring (35) substantiated his earlier findings when he found, in man, that the metabolites excreted in a 24-hr urine sample were:

(1)	Amphetamine	30%
(2)	p-hydroxyamphetamine	3%
(3)	Benzoic acid	21%
(4)	1-phenylpropan-2-one	3%
(5)	1-phenylpropan-2-ol	0%

The subjects received an oral dose of 0.07 mg/kg of ^{14}C-amphetamine sulfate. These workers stated that:

> "Human urine also contains a substance (Precursor (X)) that yields 1-phenylpropan-2-one on acid hydrolysis".

It is interesting to note that Gunne and Beckett (36,30) have shown that the fate of the two optical isomers, d- and l-amphetamine are different. Beckett (30) states that:

> "Assuming excretion is the same for both isomers, the results suggest that the metabolism of l-amphetamine was less extensive than the d-isomer".

(a) Benzyl-methyl-ketone was not found free in the urine of man but as a substance which was termed "Precursor (X)" (34).

This is in agreement with the work done by Alles in 1961 (37).

Since the amount of amphetamine excreted in man is highly sensitive to the urinary pH, more being excreted in an acid than in an alkaline urine (30,38,39), the convenience of urinary studies has been questioned to some extent (40).

The relationship between urinary pH, and the extent of amphetamine reabsorption from the kidney tubules has been calculated by Beckett and others (41,42) using an analog computer to predict urinary and body levels of amphetamine when there is no urinary pH control. It is quite obvious that neither acidic urine studies nor computational methods are convenient in frequent or large scale clinical studies. In order to overcome this problem, Rowland (40) has developed a specific method for the determination of amphetamine in whole blood and urine.

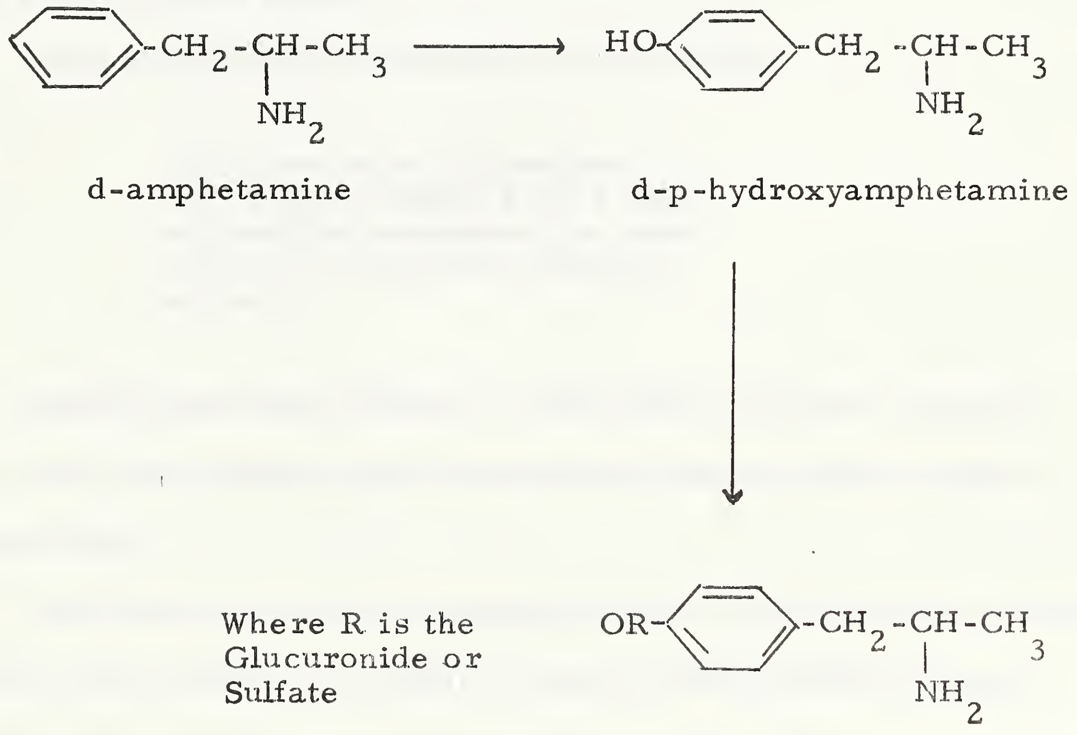
Studies of the metabolic fate of amphetamine in dogs, rats, guinea pigs and rabbits were reported by Axelrod in 1954 (43). The following results for the biotransformation of amphetamine were reported.

Thirty-one percent of a 5 mg/kg dose of d-amphetamine administered to dogs was excreted unchanged in the urine over a period of 48 hours. Twenty percent of the dose was recovered as p-hydroxyamphetamine (Figure I).

Hydroxylation of amphetamine was also observed in rats. Animals given a 5 mg/kg dose of d-amphetamine excreted 13% of the drug in the parent form and thirty-one percent as the hydroxy compound, but hydroxylation of amphetamine was not observed in rabbits or guinea pigs after an intraperitoneal injection of 5 mg/kg of d-amphetamine.

Figure I

A diagram of the metabolic transformation of d-amphetamine in the dog



To delineate further the intermediate metabolism of d-amphetamine the fate of d-p-hydroxyamphetamine was examined in dogs, guinea pigs, rats and rabbits (43). After the intraperitoneal administration of 5 mg of d-p-hydroxyamphetamine hydrobromide dogs, rats, guinea pigs and rabbits excreted 61,34,69 and 55 percent of the drug respectively, in both the free and conjugated forms, showing that the compound is relatively stable in these species. The remainder of the d-p-hydroxyamphetamine hydrobromide was not accounted for by the author in this paper.

From these results Axelrod concluded that:

> "Rats and dogs hydroxylate amphetamine while guinea pigs and rabbits transform the compound through other metabolic pathways".

Axelrod described further in 1955 (44) an enzyme system in rabbit liver microsomes which deaminates amphetamine to yield phenylacetone.

This ketone was shown in vivo by Smith and Williams, in 1954, to yield 1-phenylpropan-2-ol (45). These workers further demonstrated in 1956 (46) that benzyl-methyl-ketone is in fact metabolized to 1-phenylpropan-2-ol and benzoic acid, while the alcohol is partly oxidized to benzoic acid and partly conjugated with glucuronic acid. The main pathways of amphetamine metabolism (excluding conjugation) could thus be expressed in Figure 2.

Alleva in 1963 studied the metabolism of ^{14}C-dl-amphetamine in the rat (47). From this work he identified hippuric acid as a metabolite of amphetamine. This shows that the rat can deaminate

amphetamine. The microsomal enzyme system responsible for deaminating amphetamine was therefore found to be present both in rabbit and rat. Alleva, because he could not find benzoic acid, speculated in his paper that the

> "Phenylacetone is metabolized to hippuric acid in the body and that the small amount of hippuric acid ^{14}C recovered in the present study may be due to the low enzyme activities observed by Axelrod in rat liver as compared with rabbit liver. The present findings are in agreement with the view that the main metabolic pathway of amphetamine in the rat is not through deamination as in the rabbit but through ring hydroxylation with subsequent formation of the glucuronide".

The major portion of the detected radioactivity was suspected to be this conjugate derivative.

Noujaim in 1964 found that the metabolites of ^{14}C-amphetamine sulfate in the rat were p-hydroxyamphetamine, benzoic acid, hippuric acid, and other unidentified compounds. He did not find the glucuronide or other conjugates of the products (48).

Dring and others (35) reported that the urinary metabolites of amphetamine sulfate in the rat were amphetamine, p-hydroxyamphetamine and benzoic acid. They did not find hippuric acid. In the rabbit they found amphetamine, p-hydroxyamphetamine, benzoic acid, 1-phenylpropan-2-one(a), 1-phenylpropan-2-ol, and in the dog

---

(a) The suggestion has been made, from experiments done on rabbit urine, that the precursor of 1-phenylpropan-2-one might be the enol sulphate of the ketone (35).

amphetamine, p-hydroxyamphetamine, benzoic acid, 1-phenylpropan-2-one and 1-phenylpropan-2-ol. They also agreed with Alleva when they stated that aromatic hydroxylation is a major metabolic reaction of amphetamine in the rat (33).

Ellison and others (49) have reported the metabolites of amphetamine sulfate in the dog to be, the glucuronide of p-hydroxyamphetamine, p-hydroxy-d-amphetamine and hippuric acid. Their studies on the monkey yielded p-hydroxy-d-amphetamine and hippuric acid as the metabolites.

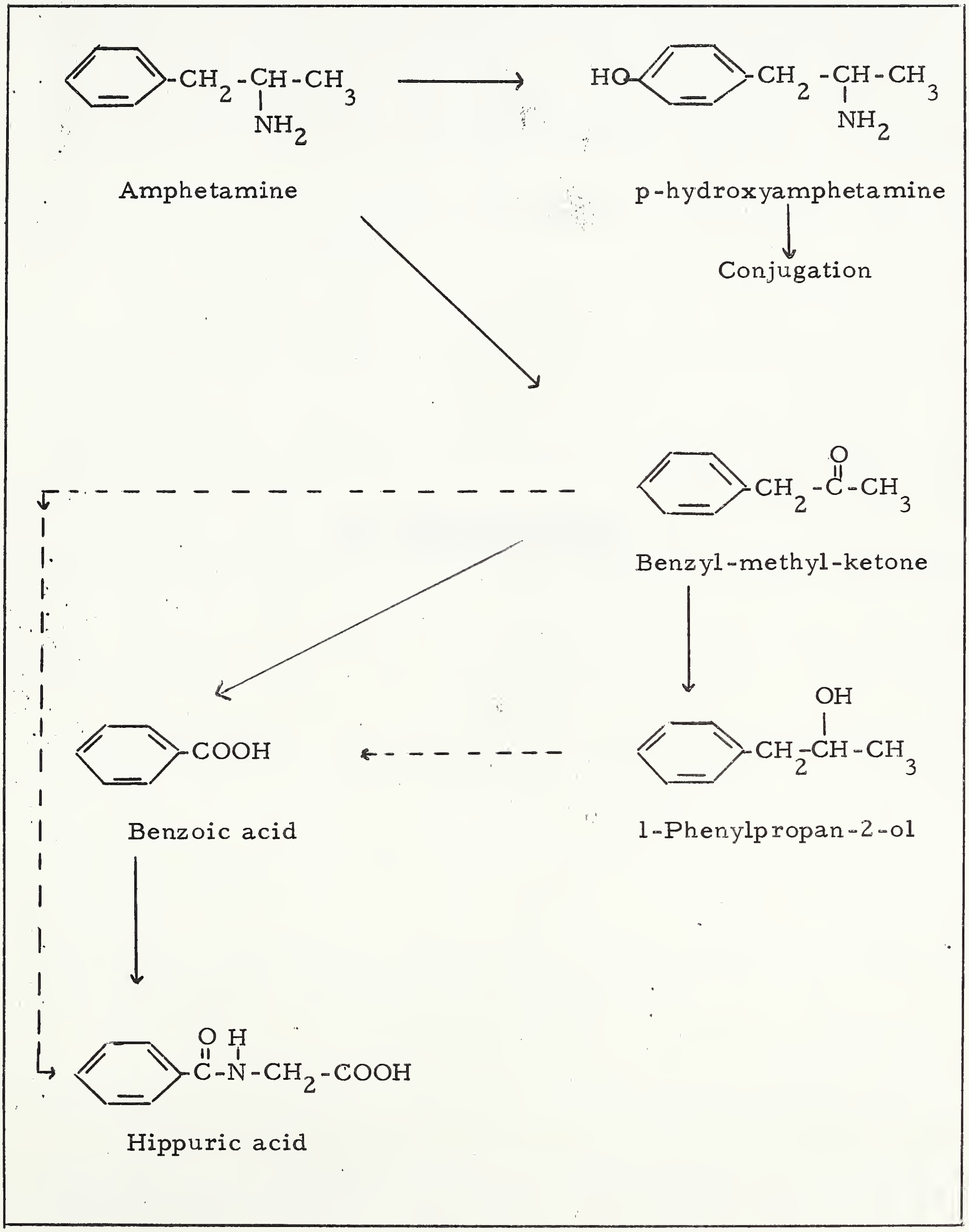
In 1968 Ellison and others (50) studied the metabolites of amphetamine HCl in the cat. They used ^{3}H dl-amphetamine HCl and extracted the metabolites from a 24 hour urine sample. The average percentage of each metabolite was reported to be:

Unchanged amphetamine	61.2%
Hippuric acid	13.9%
Hydroxyamphetamine and its glucuronide	21.7%
Phenylpropanolamine (Nor-ephedrine)	3.2%

Since cats are known to be deficient in their conjugation systems, the above results seem to be quite doubtful (54).

Figure 2

Diagram of the proposed pathways of
amphetamine metabolism
in several species



III. EXPERIMENTAL

A. Purity Determination of the Standard

The ^{14}C-labeled amphetamine sulfate used in this study was obtained from the Smith Kline and French laboratories. To determine its purity the material was analysed by thin layer chromatography.

A standard solution was prepared having a specific activity of 4.3×10^4 dpm/µl. This was subjected to thin layer chromatography in a solvent system of cyclohexane, chloroform and diethylamine in the ratio of 5:4:1 (48).

The plate was coated with silica gel G and autoradiography was used to detect the number of spots. The plate was allowed to develop for two weeks. Three minor impurities and one major impurity were detected on the X-ray film. A template of the X-ray film was made, and placed over the silica gel plate. Using a pin, the main spot corresponding to amphetamine was outlined on the plate. The template was removed and the spot was scraped using a small stainless steel spatula into a small "scoop" made from aluminum foil. Using this "scoop" the material was transferred quantitatively into a scintillation vial containing 3% of Cab-O-Sil(a) in a Toluene fluor. The vial was then counted in the Nuclear Chicago model 720 liquid scintillation spectrometer(b). The amount of impurity detected was found to be 7.3% of the total activity. Thus it was decided that the material was sufficiently pure to be used in this investigation.

(a) Cab-O-Sil Cabot Corp., 125 High Street, Boston, Mass., U.S.A.

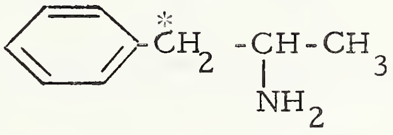
(b) Nuclear Chicago Corp., 333 East Howard Avenue, Des Plaines, Illinois, 60018, U.S.A.

B. Preparation of the Animals

Five male cats were used in this experiment. The animals were all approximately of the same age and in good health. They were obtained at least a week or two before being used in order for them to become accustomed to the laboratory as well as to being handled. They were given soft cat food twice daily and water ad libitum. The animals were exercised daily.

A large metabolism cage was used to confine each animal during the experiment. A stainless steel drip pan was placed under the entire bottom of the cage to collect the urine samples.

Each cat was injected intraperitoneally with a 5 mg/kg dose of labeled d-amphetamine sulfate having a specific activity of 2 μCi/mg, the position of the label being as indicated in the following structural formula:



$$C_6H_5-\overset{*}{C}H_2-\underset{NH_2}{\underset{|}{CH}}-CH_3$$

C. Collection and Processing of the Urine Samples

Urine samples were collected from each cat every 24 hours for four days. Table II shows the volume and the pH of the urine collected from each animal.

TABLE II

Volume and pH Values of Cat Urines after d-amphetamine Administration

	Time of Collection			
	24 Hr	48 Hr	72 Hr	96 Hr
Cat A. Vol pH	97 ml 6.5	125 ml 6.6	105 ml 6.4	110 ml 6.5
Cat B. Vol pH	52 ml 6.8	83 ml 6.7	67 ml 6.3	73 ml 6.4
Cat C. Vol pH	43 ml 6.3	70 ml 6.4	53 ml 6.3	80 ml 6.1
Cat D. Vol pH	36 ml 6.8	130 ml 6.7	79 ml 6.6	90 ml 6.7
Cat E. Vol pH	40 ml 6.4	23 ml 6.6	26 ml 6.4	51 ml 6.5

The urine samples were centrifuged at 1,000 rpm for 30 minutes immediately after collection and then frozen. Upon the completion of each animal experiment the four urine samples obtained were then freeze-dried, and reconstituted in 10 ml of distilled water. A part of each urine sample was then taken and counted according to the following procedure. For cats A, B, and C 100 µl of each urine sample was placed in a scintillation vial. Fifty microliters of 50% hydrogen peroxide was added and the vial was allowed to stand at room temperature for twelve hours, in order to allow for decomposition of the

hydrogen peroxide. Fifteen millilitres of fluor were added and the samples were counted for ten minutes in the Nuclear-Chicago model 720 liquid scintillation spectrometer. The fluor used was as follows:

Dioxane	600 ml
Anisole	100 ml
Dimethoxyethane	100 ml
PPO	12 g
POPOP	500 mg

Since this did not appear to be a very satisfactory procedure, several different fluors were tried. From this, it was decided that the following method be used to count all of the urine samples.

A large volume of toluene-butyl PBD-PBBO fluor(a) was prepared. Twenty millilitres of this mixture was placed into a scintillation vial. Three drops of 4% $SnCl_2$ in 0.1 N HCl, and 200 µl of BBS-3 Bio Solv solubilizer(b) were also added to each sample. One hundred µl of the reconstituted urine sample was mixed directly with the above cocktail. The vial was capped and shaken well until a clear solution resulted.

A set of standards was prepared using the above fluor. The standards used contained 0, 40, 80, 100, 140, 180, 220, and 240 µl of "cold" urine sample. To each standard was added a measured amount of radioactivity equivalent to 322,000 dpm of ^{14}C-amphetamine sulfate.

The urine standards were counted before and after the urine samples, and quench correction was effected by means of the

(a) Fluoralloy, Formula TLA (Beckman #161233)

(b) Bio-Solv solubilizer (BBS-3) (Beckman #184983)

channels' ratio technique. Each sample was counted in a liquimat model 220 liquid scintillation spectrometer* for 10 minutes.

A 5 ml aliquot of each urine sample was taken and divided into two 2.5 ml portions. One 2.5 ml portion was hydrolyzed by the addition of concentrated HCl to a pH of 2, followed by heating for 2 hours at 75°C on a water bath. The other 2.5 ml portion was set aside.

Two series of thin layer plates were prepared using silica Gel G. One set was 0.25 mm and the other was 1 mm thick. Four 10 µl aliquots of urine sample and a standard 10 µl volume of ^{14}C-amphetamine sulfate were placed on the 0.25 mm plates. On the 1 mm plates was streaked one ml of urine sample, and a 10 µl spot of ^{14}C-amphetamine standard. This procedure was effected for both hydrolyzed and non-hydrolyzed urine samples.

D. Collection and Processing of Blood Samples

Blood samples were taken from each animal at 15 min, 30 min, 1 hour, 6 hours, 12 hours, and 24 hour intervals. For cats A, B, and C, these samples were taken from the jugular vein using a vacutainer(a) which contained 9 mg of ethylene diamine tetraacetic acid. The vacutainer needles used were monoject (210) 22 gauge x 1 1/2 in. For cats D, and E, the blood samples were obtained by cardiac puncture, using a 10 ml glass syringe (heparinized) and a 22 gauge x 1 1/2 in

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*Liquimat Model 220 Liquid scintillation spectrometer manufactured by Intertech, Inc., 333 State Street, North Haven, Connecticut, U.S.A. an affiliate of Picker Nuclear, 1275 - Mamaroneck Avenue, White Plains, New York, 10605, U.S.A.

(a) Vacutainer - B-D. Vacutainer #3204 Manufactured by Becton-Dickinson and Co., Canada, Ltd., Clarkson, Ontario.

needle. The cardiac puncture technique was found to be superior to that of the vacutainer procedure as it was much quicker and easier.

The blood samples were centrifuged in vacutainer tubes at 2,000 rpm for 30 min immediately after collection. The blood samples were then frozen until they were counted.

Two counting procedures were studied. The first was as follows:

(1) 25 µl of plasma was placed in a scintillation vial.

(2) 50 µl of hydrogen peroxide was added.

(3) The vial was left at room temperature for twelve hours.

(4) 200 µl of N.C.S.[a] reagent was added and the vial was again left at room temperature for twelve hours.

(5) 15 ml of fluor[b] was added and the samples were counted in the Nuclear-Chicago liquid scintillation spectrometer model 720 for 10 minutes.

The above method was found to produce phase separation in some samples and was thus judged unsuitable.

The second procedure consisted of adding 25 µl of plasma to the cocktail mixture used for counting the urine samples. Each vial was capped and shaken well until a clear solution resulted. Each

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[a] N.C.S. reagent is a mixture of bases in the molecular weight range of 250-600 produced from a commercial mixture of chlorides of the type formula $R_2 R'_2 NCl$ where R is methyl and R' is a straight chain varying from C_6 to C_{20}, with an average of about 12 carbons. The product is produced by Nuclear-Chicago Corporation,

[b] The fluor used was: Dioxane 600 ml, Anisole 100 ml, Dimethoxyethane 100 ml, PPO 12 g, POPOP 500 mg.

sample was then counted in a Liquimat model 220 liquid scintillation spectrometer for 10 minutes.

A set of quenched standards was prepared using the above fluor. The standards used contained 0, 25, 50, 100, 200, 300, 400, and 500 μl of "cold" plasma sample. To each standard was added a measured amount of radioactivity equivalent to 322,000 dpm of ^{14}C-amphetamine sulfate having a specific activity of 6 μCi/mg. The plasma standards were counted before and after the plasma samples and quench correction was effected by means of the channels' ratio technique.

Table 3 shows the results of urinary excretion of ^{14}C-amphetamine and its metabolites. It was observed that the maximum urine level was obtained 48 hours after the intraperitoneal injection of the drug. Figure 3 is a graphic representation of the above data.

Table III

The urinary excretion of ^{14}C-amphetamine and its metabolites observed in the cat over a four-day period.

Sample	Total Activity* Excreted (dpm)	s.d. %	Cumulative[(a)] Excretion %
Day-1	6.43×10^7	±14.92	21.61
Day-2	1.26×10^8	±16.74	63.90
Day-3	4.29×10^6	±10.01	65.76
Day-4	1.45×10^6	±14.94	66.69

*Figures shown are the averages of four cats.

(a)Total excretion measured.

Figure 3

Excretion of ^{14}C-amphetamine and its metabolites in the urine

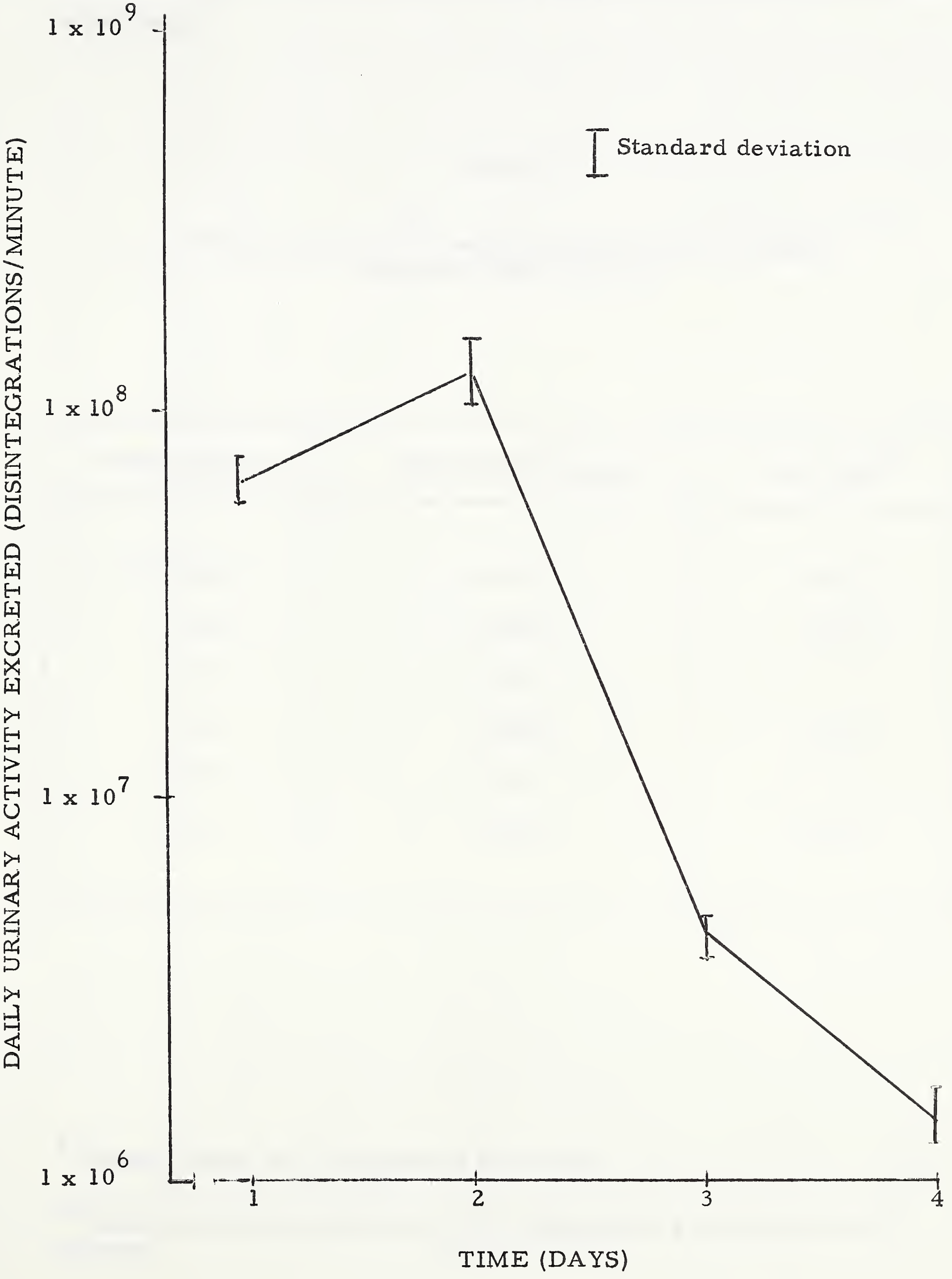


Table IV and figure 4 depict the levels of ^{14}C-amphetamine and its radioactive metabolites in the plasma of the cats used in this experiment.

Table IV

Plasma levels of ^{14}C-amphetamine in the cat after intraperitoneal injection.

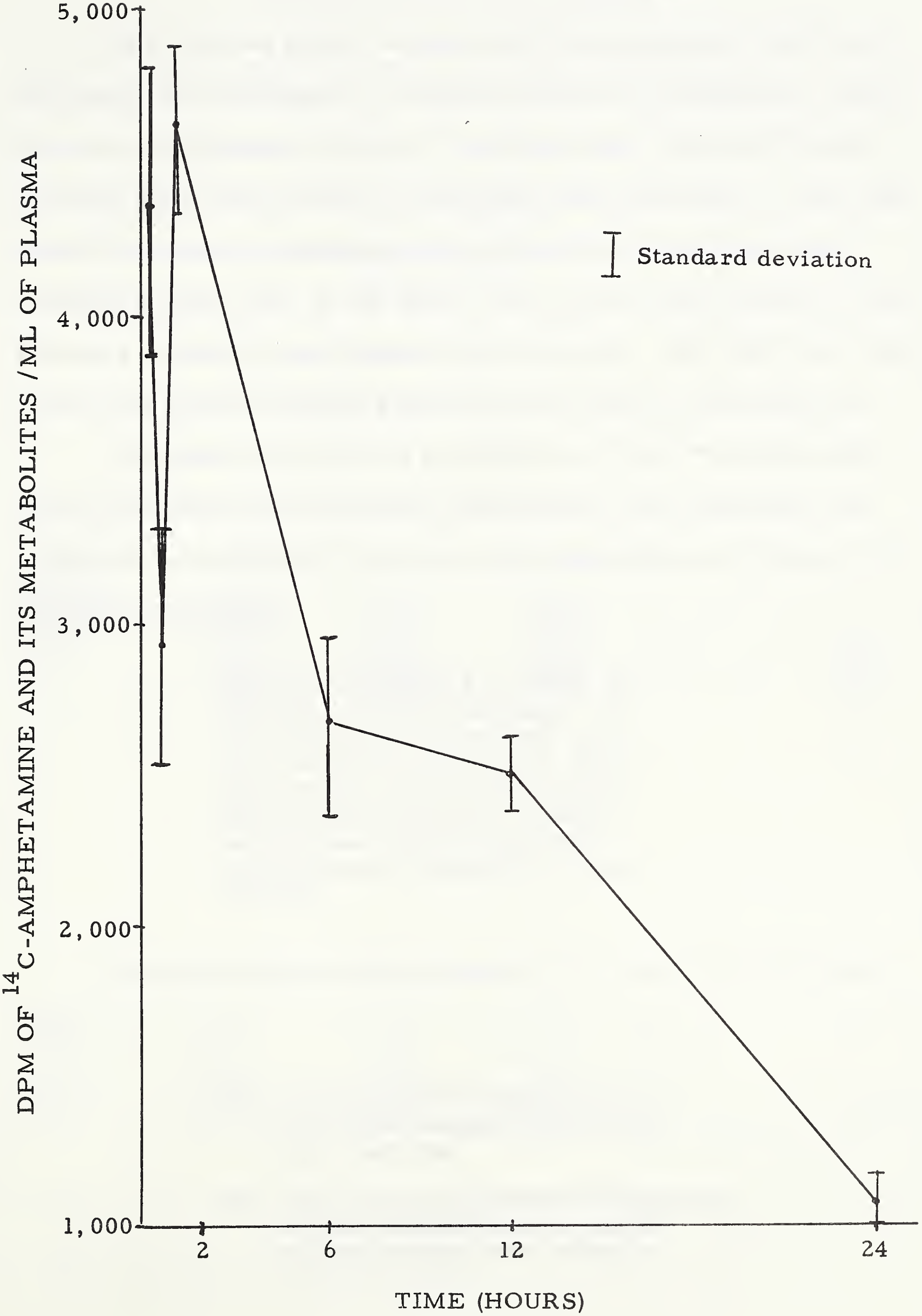
Time Interval	dpm/ml of Plasma*	s.d. (%)(a)
15 min	4,274	± 12.8
30 min	2,942	± 13.7
1 hr	4,631	± 7.3
6 hr	2,642	± 12.6
12 hr	2,511	± 4.2
24 hr	1,109	± 10.1

*Figures shown are averages of four cats.

(a)Standard deviation based on the radioactivity present per ml of plasma.

Figure 4

Plasma levels of ^{14}C-amphetamine and its radioactive metabolites in the cat



E. Separation of Urinary Metabolites

When the two series of plates had been prepared, each 8 x 11 - inch plate was developed in a solvent system of cyclohexane, chloroform and diethylamine (5:4:1:). The plates were removed from the tank and dried with the use of a portable hand hair dryer. Once they were thoroughly dry each plate was placed in a 8 x 12 inch Kodak cardboard folder and, in the dark, a no-screen Kodak medical X-ray film was carefully superimposed onto the plate. The folder was then closed and a piece of glass placed over the top for even pressure.

The plates were left for a period of at least two weeks after which time they were developed using Kodak X-ray developer and replenisher, and Kodak X-ray fixer and replenisher according to the following procedure:

> The film was placed in the X-ray developer for four minutes. It was then taken and washed in the water bath for one minute, after which it was placed in the X-ray fixer for ten minutes. Finally, the film was transferred to a water bath and washed for twenty minutes.

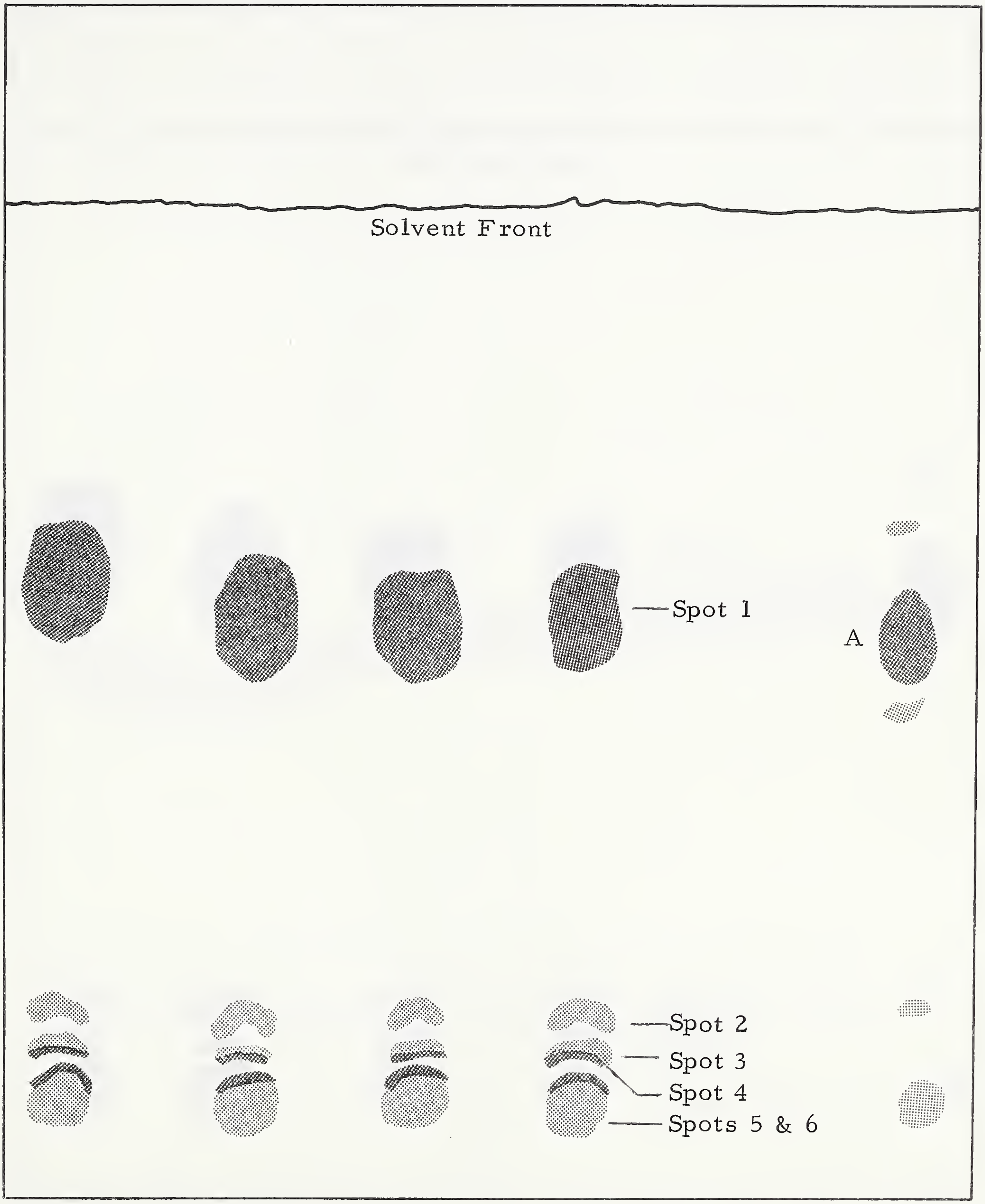
From the figures shown (Figures 5, 6, and 7), it can be seen that:

(1) Six spots were observed with the origin possibly containing more than one.

(2) There was no qualitative difference between the hydrolyzed and the non-hydrolyzed urine samples.

Figure 5

Diagram of the autoradiogram obtained from a non-hydrolyzed 48-hr urine sample



A ^{14}C-amphetamine sulfate standard

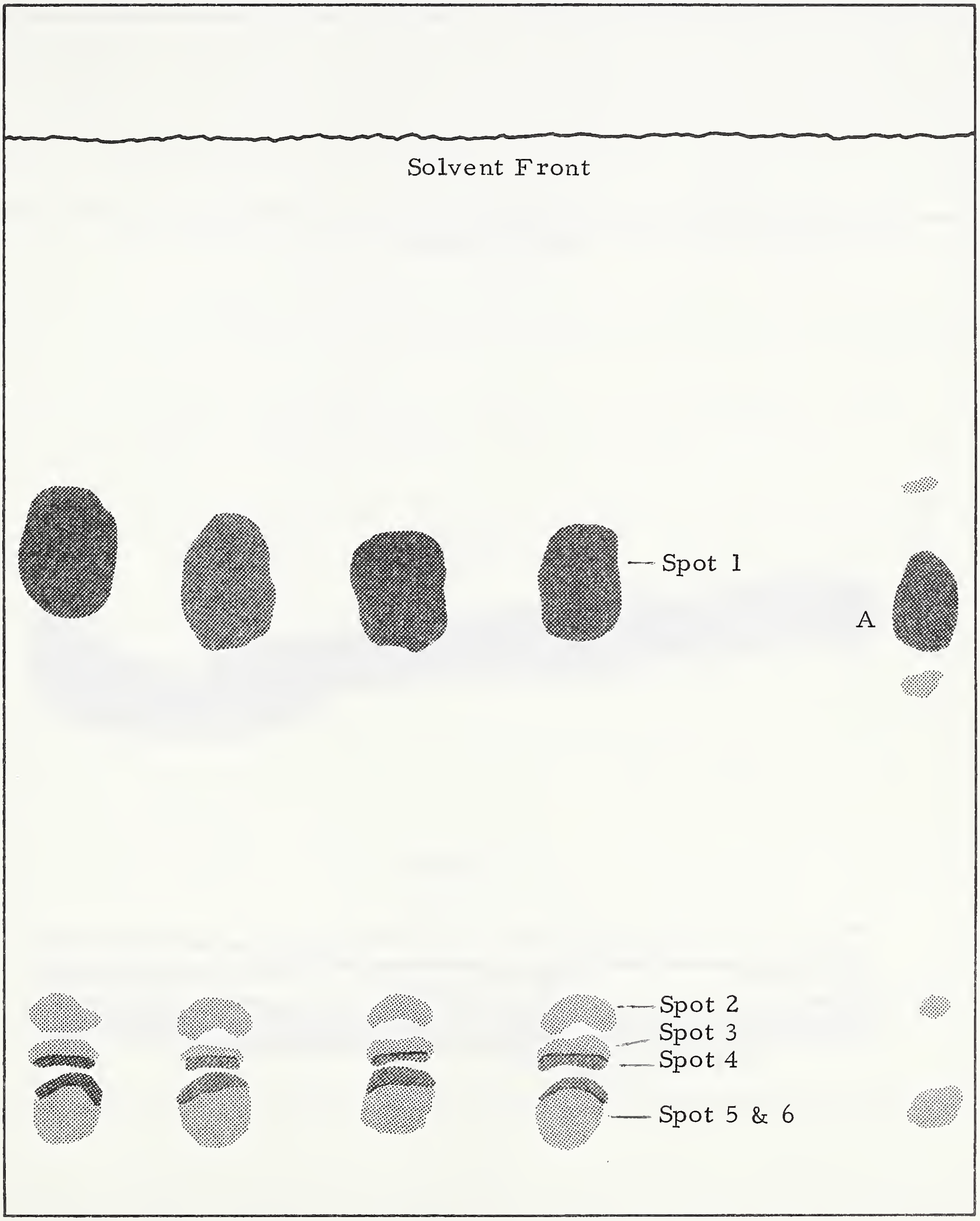
[illegible]

[illegible]

[illegible]

Figure 6

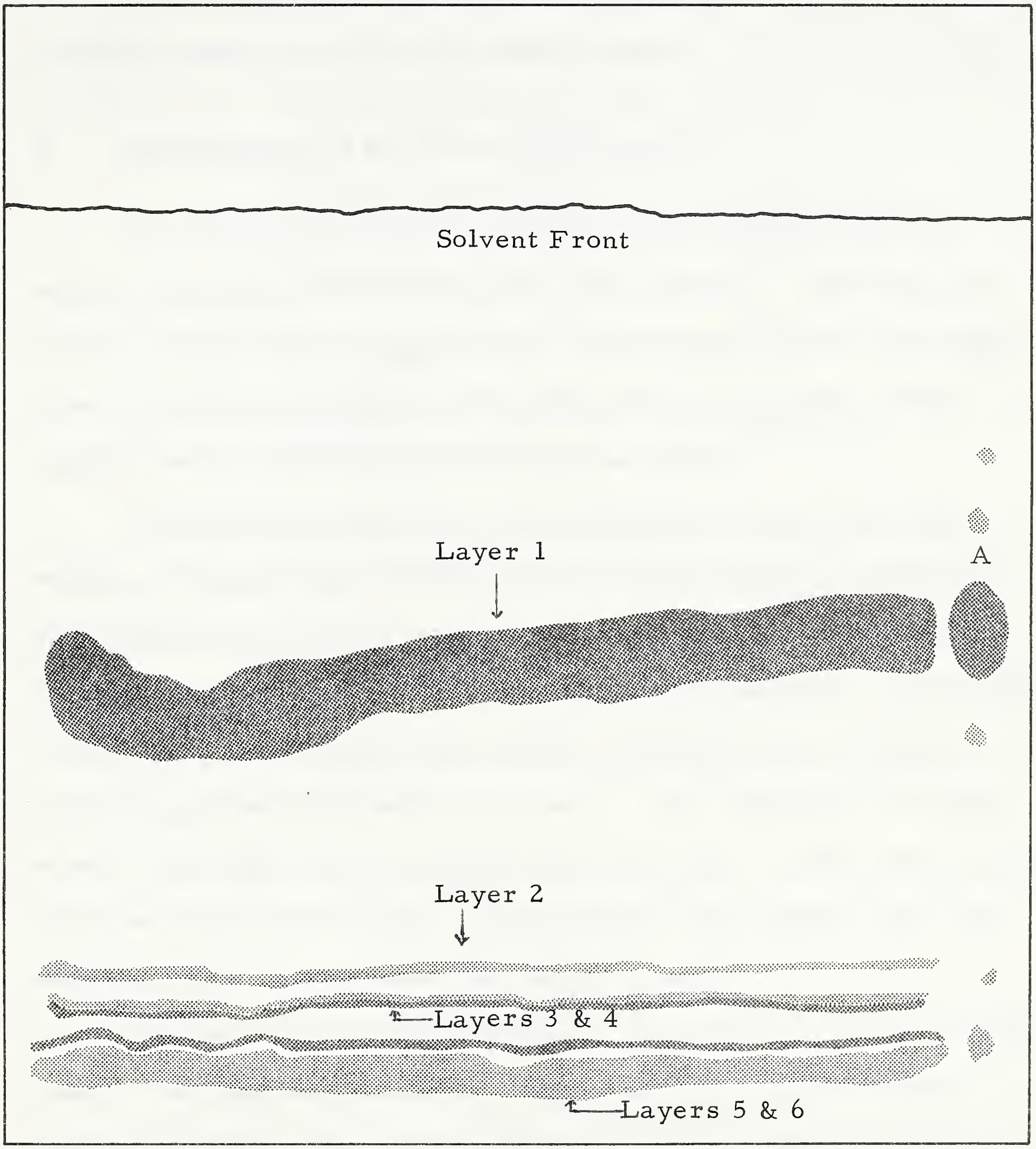
Diagram of the autoradiogram obtained from a 48-hr hydrolyzed urine sample



A ^{14}C-amphetamine sulfate standard

Figure 7

Diagram of a typical autoradiogram obtained from a preparative TLC plate of a 48-hr urine sample



A ^{14}C-amphetamine sulfate standard

(3) The six possible spots were clearly
visible on the autoradiograms
obtained from the preparative plates.

The various spots were given the numbers 1, 2, 3, 4, 5, and 6 in a descending order from the solvent front.

F. Identification of the Urinary Metabolites

An attempt was made to identify the metabolites following solvent extraction directly from the urine samples. This was performed using "cold" d-amphetamine sulfate and injecting a cat with a dose of the drug equivalent to 10 mg/kg intraperitoneally. Urine samples were collected every 24 hours as before.

The extraction procedure was attempted on the 24-hr urine sample. Seventy-five millilitres of the urine sample were filtered after adjustment to pH 7, placed in a 250 ml separatory funnel, and washed five times with 67.5 ml portions of ethyl acetate. An emulsion resulted and 30 ml of Iso-amyl alcohol was added in an attempt to break the emulsion but with no success. One millilitre of absolute ethanol was added and the emulsion left overnight at which time only about half had separated out. Half-saturated salt solution was then added and a clear separation was finally obtained.

All the washings were combined and placed in a 500 ml boiling flask. The flask was placed on a flash evaporator and the volume reduced to 25 ml. The salt was then removed by chloroform extraction. When the chloroform was added, however, an emulsion formed again. After standing overnight the emulsion had separated and the chloroform fraction was placed in a small boiling flask and reduced to a volume of 0.5 ml.

Gas chromatography of the chloroform extract was then attempted using an F. and M. model 500 gas chromatograph.(a)

A stainless steel six foot, one-eighth inch o.d. chromatographic column packed with 10% apiezon L on 60 - 80 mesh diatoport S was used. Operating conditions were as follows:

Column Temperature	250°C
Injector Temperature	300°C
Detector Temperature	275°C
Helium Pressure	21 lbs./sq.in.

Flow Rate:

Detector Exit	60 ml/min
Reference Exit	25 ml/min

Ten to thirty μl of each sample were injected and a peak was obtained with a retention time of 10 minutes. As the sample eluted from the column it was collected in a capillary tube and a thin film IR spectrum was attempted using the sample collected. This, however, was unsuccessful. No other peaks appeared on the chromatogram after 30 minutes.

An acidic extraction was then attempted. The remaining 75 ml of the 24 hr urine sample was placed in a separatory funnel, and 6 N HCl was added until a pH of 2 was obtained. The urine was washed three times with 37.5 ml portions of redistilled ether. After evaporation to a volume of 0.5 ml the sample was injected into the F. and M. model 500 gas chromatograph as before, but with no results.

An alkaline extraction was then attempted by adding 20%

(a) F. and M. model 500 gas chromatograph, manufactured by Hewlett-Packard (F. and M. Scientific Division) Wilmington, Delaware, U.S.A.

NaOH to the above urine until a pH of 12 was obtained. The urine was washed three times with 35 ml portions of redistilled ether. The ether portion was evaporated as before to a volume of 0.5 ml. The sample was injected into the F. and M. model 500 gas chromatograph and again no results were obtained.

A further chromatographic column was used in an attempt to obtain peaks for the extracted metabolites. The column was a stainless steel six foot, one-eighth inch o.d. packed with 10% carbowax 20M on 60 - 80 mesh diatoport S(a). Operating conditions were as before except that the oven temperature was reduced to 200°C, but no peaks were obtained after 1 1/2 hours.

It was decided to try the F. and M. model 700 gas chromatograph with the following operating conditions:

Injection Port	300°C
Oven	150°C
Detector	350°C
Helium Pressure	40 lbs./sq.in.
Air Pressure	25 lbs./sq.in.
Hydrogen Pressure	15 lbs./sq.in.

The column used was a 6 foot, one-eighth inch o.d., stainless steel packed with OV. -17(a) silicone gum rubber on 80 - 100 mesh AW/DMCS treated Chromosorb G, but again no peaks were obtained.

Since this method was time consuming as well as appearing impractical for this work, it was decided to attempt to identify the metabolites by using the preparative TLC plates prepared from the 48-hr urine samples.

(a) Diatoport S, Chromosorb G., Ov -17 silicone gum rubber, manufactured by Hewlett-Packard Co.

Layer 1, which from its R_F value was suspected to contain unchanged d-amphetamine sulfate was partially removed and placed in a small fine porosity sintered glass funnel. Seventy percent alcohol was poured through the funnel under vacuum and the filtrate was collected. The filtrate was evaporated to dryness on a flash evaporator and a brownish powder was obtained. An infra-red spectrum was taken of this powder using a micro-pellet technique. A standard of d-amphetamine sulfate was prepared by mixing the latter with a portion of silica gel obtained from the same plate, extracted with 70% alcohol, and evaporated to dryness.

The spectra obtained for the standard and for the sample appeared to be the same. It was, therefore, assumed that layer 1 was d-amphetamine sulfate. To be certain, however, layer 1 was also treated as follows: The silica gel powder was shaken with 5 ml of 1 N H_2SO_4 then filtered through a suction tube. The material remaining in the sintered glass funnel was washed with two 5 ml portions of distilled water and refiltered. Both filtrates were combined. The latter was mixed with 22 ml of 30% sodium hydroxide and 0.5 g of d-amphetamine sulfate. The mixture was shaken vigorously then cooled with dry ice. Benzoyl chloride reagent was added dropwise while shaking vigorously. The vial was cooled until the odor of the reagent persisted.

The precipitate formed was filtered by suction and dried between two pieces of filter paper. It was then re-crystallized four times from boiling 70% alcohol. A sample of the crystals obtained after each crystallization was weighed and dissolved in 95% ethyl alcohol. One ml of the final solution was added to 15 ml of prepared

Beckman fluor and counted in the liquimat model 220 scintillation spectrometer. The results are given in table V.

Table V

Identification of Amphetamine Sulfate as a metabolite using Isotope dilution analysis

Crystallization #	Sample Weight	Activity cpm/mg
1	20 mg	149.8
2	20 mg	151.1
3	20 mg	155.5
4	20 mg	152.5

It was therefore concluded that layer 1 is unchanged d-amphetamine sulfate, since the specific activity remained constant after several crystallizations.

A portion of layer 2, suspected to contain p-hydroxyamphetamine, was taken from the preparative plate and extracted similarly to layer 1. An infra red spectrum of the white powder obtained was taken. Another spectrum containing standard p-hydroxyamphetamine hydrobromide was observed for a sample prepared in an identical manner as before.

From the similar IR spectra it was suspected that this layer contained p-hydroxyamphetamine. As a final method of identification

isotope dilution analysis was done as follows:

The silica gel powder was mixed with 500 mg of sodium carbonate and the mixture was shaken with 5 ml of distilled water. The suspension was filtered by suction and the material remaining in the filter was washed with an additional 5 ml of distilled water and re-filtered. The filtrates were combined and extracted with two 10 ml portions of diethyl ether. In the aqueous solution, about 800 mg of p-hydroxyamphetamine HBr, 500 mg of sodium carbonate, and 1 g of potassium carbonate were dissolved. The latter solution was then extracted with four 10 ml portions of ether. The aqueous solution was re-shaken with the extracted silica gel and filtered by suction. The filtrate was extracted with a further 10 ml portion of ether. All ethereal extracts were combined and evaporated to dryness on a flash evaporator. The remaining residue was re-crystallized from benzene three times. A 20 mg sample was taken after each crystallization and dissolved in 95% ethanol. One ml of the latter solution was added to 15 ml of prepared Beckman fluor and counted in the liquimat model 220. The results are given in table VI.

Table VI

Identification of p-hydroxyamphetamine as a metabolite
using Isotope dilution analysis

Crystallization #	Sample Weight	Activity cpm/mg
1	20 mg	198.3
2	20 mg	210.6
3	20 mg	201.8

It was therefore concluded that layer 2 is p-hydroxyamphetamine.

Layers 3 and 4 were first suspected to contain either benzoic and/or hippuric acid from the infra red spectrum which had been done in the same manner as described before.

To determine if benzoic and/or hippuric acid was present layers 3 and 4 were treated as follows:(a)

The gel powder was boiled in a beaker with 10 ml of 95% ethyl alcohol and filtered through suction while hot. The material remaining on the filter was washed with another 5 ml portion of boiling alcohol.

Four hundred mg of benzoic acid were dissolved in 7 ml of the filtrate, and the solution was then mixed with 7 ml of distilled water. The final solution was heated to boiling and then left to crystallize. Three repeated crystallizations were effected using aqueous ethyl alcohol. A sample was taken after each crystallization, dissolved in 95% alcohol and counted in the liquimat model 220 liquid scintillation spectrometer as before.

In the remaining portion of the original alcoholic extract of the gel powder, 400 mg of hippuric acid were dissolved. The solution was heated to boiling and left to cool slowly in a refrigerator to help crystallization. Three crystallizations were effected from aqueous alcohol. Samples were taken after each crystallization as before and counted in the liquimat model 220 liquid scintillation spectrometer.

Constant specific activity was not obtained from this procedure, for either benzoic acid or hippuric acid. It was therefore concluded that layers 3 and 4 did not contain benzoic or hippuric acid.

(a) Because of the close proximity of layer 4 to layer 3, layer 4 was removed quantitatively with layer 3.

Since this layer did not change in position on the autoradiogram when the urine sample was hydrolyzed, when compared to the non-hydrolyzed sample, it is believed that this metabolite is not a conjugate.

Since no concrete evidence as to the exact identity of this metabolite (or metabolites) was found, the nature of these layers (layers 3 and 4) on the autoradiogram remains unsolved.

Because of the close proximity of layer 5 to the origin (layer 6) layer 5 was removed quantitatively with layer 6, and placed in a small fine porosity sintered glass funnel. Seventy percent alcohol was poured through the funnel under vacuum and the filtrate was collected. The filtrate was evaporated to dryness in a flash evaporator. A brownish liquid was obtained.

The residue was suspected to be a mixture of several metabolites. An attempt was made to separate these metabolites by the following procedure.

The residue was dissolved in 0.5 ml of water, and 10 μl spots were placed on strips (3 cm. x 20 cm.) of Chrom AR 500 chromatographic paper. The strips were developed in a solvent system of Benzene: Dioxane: and Acetic acid (90:25:4).

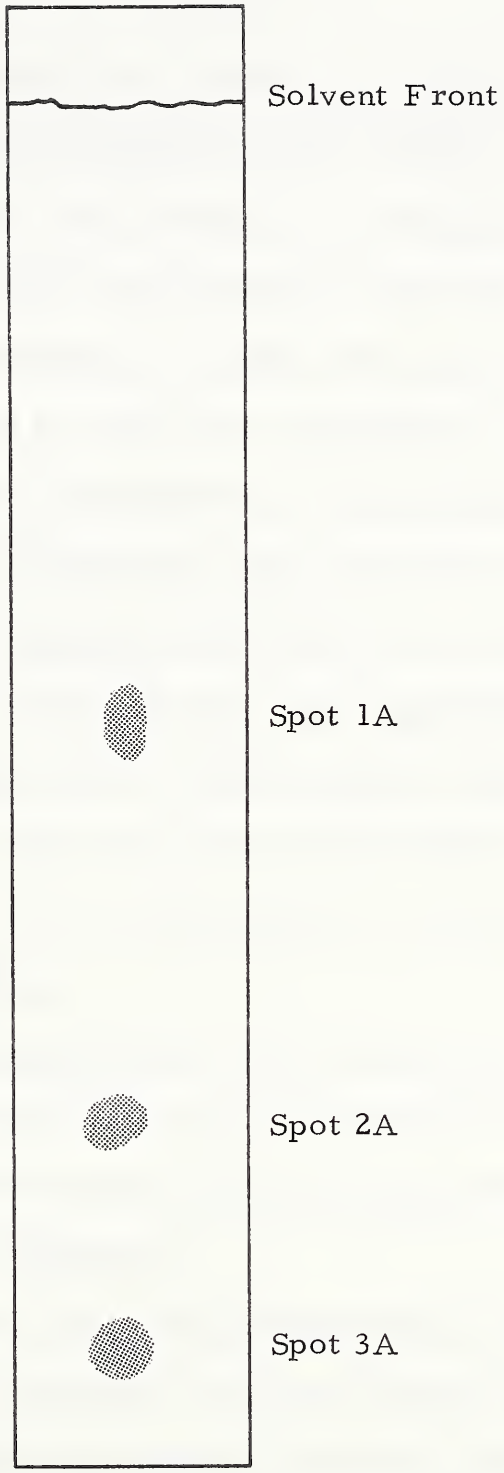
Figure 8 shows that three spots were observed on the paper, after exposure to ultra violet light. The R_F values of the observed spots in descending order from the solvent front were:

Spot 1	0.52
Spot 2	0.15
Spot 3	Origin

Standards of hippuric acid, benzoic acid, and nor-ephedrine were also spotted on Chrom AR 500 paper, developed in the above solvent system, and observed under the ultra violet light. The R_F values of the standards

Figure 8

Separation of Layers 5 & 6 on thin-layer chromatographic paper. (Chrom AR-500)



were:

Hippuric Acid	0.50
Benzoic Acid	0.74
Nor-ephedrine	0.12

Layers 5 and 6 were removed from another TLC plate and the procedure was repeated, with the exception that the residue was not dissolved in 1 ml of water. The latter solution was streaked across a preparative TLC plate 1 mm thick and developed in the above solvent system. Using the R_F values obtained as a guide the areas on the preparative TLC plate suspected to contain the metabolites were outlined. Figure 9 shows the relative distribution of the detected spots.

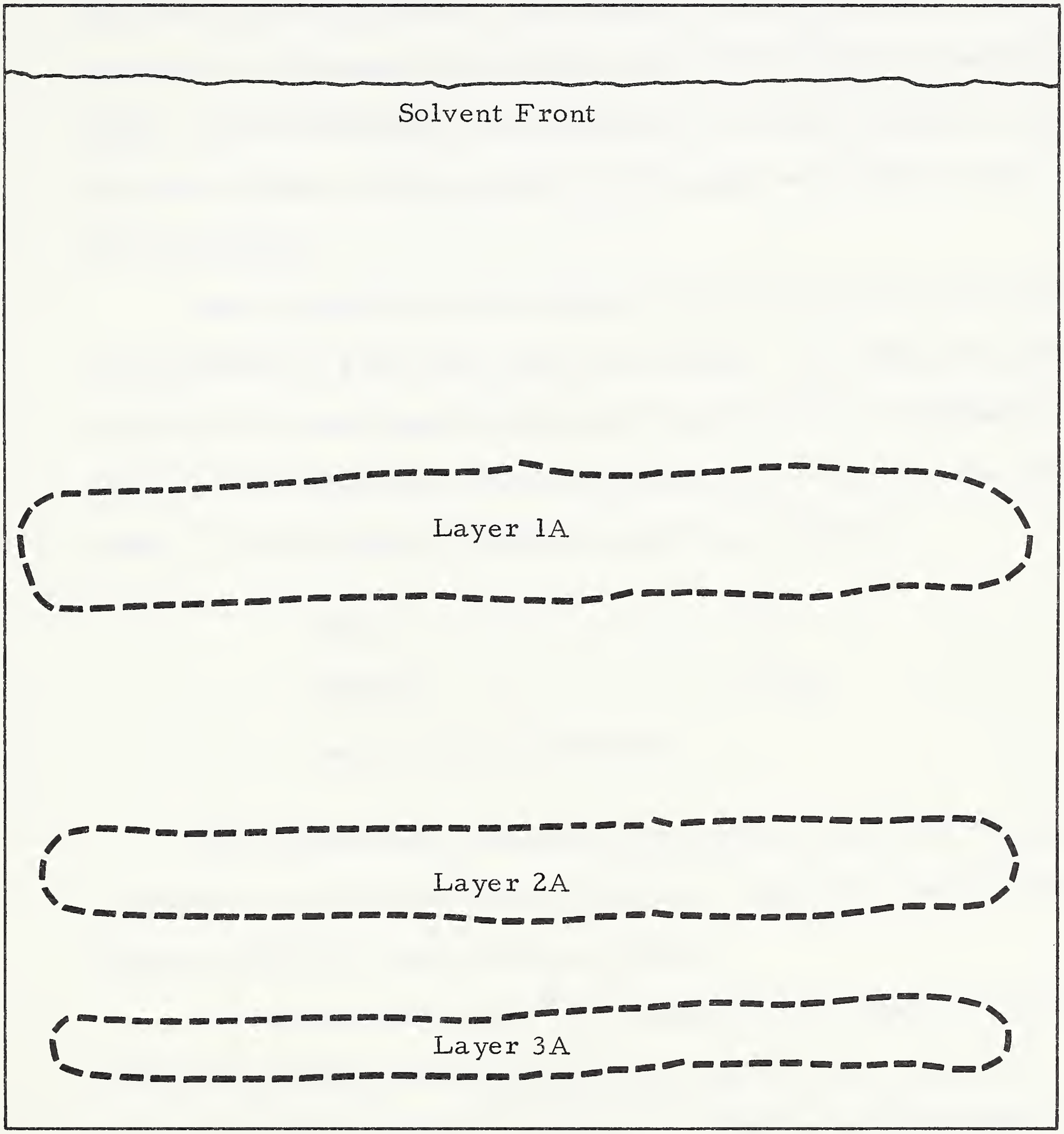
Layer 1A was removed quantitatively and extracted as before (see page 35), and an infra red spectrum was obtained in the manner described before (see page 35). From the R_F values and the infra red spectrum layer 1A was suspected to contain hippuric acid. An identification attempt was also made using the isotope dilution analysis technique but because of the small quantity of material only one recrystallization could be done.

Layer 2A was removed quantitatively and extracted as described before. An infra red spectrum was also obtained in the usual manner. From the R_F value and the infra red spectrum it appeared that layer 2A was nor-ephedrine.

The origin layer, 3A, was removed quantitatively and treated as before. No significant data, however, could be obtained from the IR spectrum. Thus, the material (materials) contained in this chromatographic layer could not be identified.

Figure 9

Diagram of the areas removed from the Preparative TLC plate - containing radioactive metabolites separated from Layers 5 & 6



G. Quantitation of Detected Metabolites

In order to determine the relative abundance of each metabolite in the urine, 10 µl samples of urine were spotted on 0.25 mm thick thin layer silica gel plates. The plates were developed in a solvent system of cyclohexane, chloroform, and diethylamine in the ratio of 5:4:1. An autoradiogram was prepared in a method similar to that described before. (See page 28). A template was made of each developed film.

Each template was then placed over the respective TLC plate and by means of a pin each spot was outlined. The different spots were then removed quantitatively and transferred to scintillation vials. Each vial was filled with Cab-O-Sil and 20 ml of toluene fluor were added. The formula for the fluor used was as follows:

PPO	4 g
POPOP	50 mg

add to 1 litre of toluene.

Each sample was counted in the liquimat model 220 liquid scintillation spectrometer for 10 minutes. Standards used for the quench correction curves were as follows:

A standard solution of ^{14}C d-amphetamine sulfate containing 322,000 disintegrations per minute was added to each vial in a series of eight vials which contained, in addition to Cab-O-Sil, 0.5 g, 1 g, 3 g, 5 g, 8 g, 10 g, 15 g, and 25 g respectively of silica gel. The silica gel used to prepare the standards was removed from areas on the developed plates that did not contain any radioactivity. Twenty millilitres of toluene fluor were added to each vial.

The results of the counting are summarized in Table VII. Appendix II gives the individual values observed for each sample examined.

Table VII

Quantitative distribution of ^{14}C-amphetamine metabolites obtained from the cat urine samples

Spot No.	Metabolite Identified	Average Excretion (%)(a)							
		24 hr	s.d%(c)	48 hr	s.d%(c)	72 hr	s.d%(c)	96 hr	s.d%(c)
1	amphetamine	53.33	14.92	45.00	17.74	5.18(b)	-	5.96(b)	-
2	p-hydroxy amphetamine	8.35	3.40	7.14	5.52	2.90(b)	-	0	-
3 & 4	unknown	4.92	3.02	6.79	4.35	28.61	13.12	42.72	13.80
5 & 6	a) hippuric acid b) nor-ephedrine c) unknown	33.29	10.30	41.10	19.62	63.51	7.40	51.38	8.83

(a) Average of five cats.

(b) Excretion in one animal only. (See appendix II Tables 3 and 4).

(c) % Standard deviation.

In order to determine the relative abundance of each metabolite on the spot of origin (Layer 5 & 6) of the 48-hour plate (See page 31) three 10 µl samples of the extract of the origin were spotted on a 0.25 mm - thick thin layer silica gel plate.

The plate was developed in a solvent system of Benzene: Dioxane: and Acetic acid (90:25:4). Using the R_F values obtained from the Chrom A.R. 500 paper (Figure 8) as a guide, the areas on the plate suspected to contain the metabolites were outlined.

Each spot was then removed quantitatively and transferred to a scintillation vial and treated as before (See page 43).

The results obtained from the counting of this plate are shown in Table VIII.

Table VIII

Relative distribution of ^{14}C-amphetamine metabolites in the spot of origin of the 48-hr urine samples

Spot	Identification	Percentage of Total Activity
1A	hippuric acid	20.02
2A	nor-ephedrine	13.75
3A	unknown	67.23

IV. DISCUSSION AND CONCLUSIONS

Amphetamine occupies a very predominant place in medicine today, with its most common use being in the treatment of obesity.

It has been estimated that eight billion amphetamine formulations are manufactured in the United States each year (25). This wide use of amphetamine in the light of its dependency-producing property has lead to the question: "Has amphetamine any real place in medicine" (51)? Swanton (52) feels that the amphetamines have little value in medicine and could be replaced by less hazardous drugs used for similar purposes.

Fulton, as stated in a paper by Bartholomew (25) feels that the amphetamines do have a place in medicine when used to treat narcolepsy, certain behaviour disorders in children often associated with abnormal EEG, certain types of epilepsy, some aggressive psychopathic conditions, and hypersomnia.

Observing the current dispute on the uses of amphetamine, and on amphetamine abuse (25, 53) it can be seen that more research is necessary to complete our understanding of amphetamine and its biotransformation in the body.

The objective of this investigation was to study the metabolism of ^{14}C-amphetamine sulfate in the cat.

It was felt that the most precise method of identification of the metabolites was one employing a thin-layer chromatographic technique coupled with autoradiography. This method offers the advantage of being extremely sensitive compared to a method such as solvent extraction coupled with gas chromatography.

To determine the quality of each metabolite it was necessary to perform thin-layer chromatography using two series of plates.

The first series of plates, 0.25 mm thick, were used to determine the relative amounts of each metabolite through the use of a liquid scintillation counting technique. The second series of plates, 1 mm thick, (Preparative plates) were used in an attempt to determine the identity of each metabolite by means of measuring the R_F values, IR spectroscopy, and isotope dilution analysis.

Using this procedure, the presence of unchanged amphetamine, p-hydroxyamphetamine, hippuric acid and nor-ephedrine was established. The identity of another three metabolites still remains unknown.

The radioactivity of both the blood and urine samples over a period of time were also determined by liquid scintillation counting.

The highest blood level appeared to occur about 1 hr following the intraperitoneal injection of the drug. This, however, was difficult to determine from the data obtained.

It was observed that the urine level reached a maximum after 48 hours, and decreased sharply in the 72 and 96 hour samples. When the relative distribution of metabolites in the urine was followed for a period of four consecutive days it was found that both amphetamine, and p-hydroxyamphetamine were predominant during the first two days after the injection of the drug. On the other hand, spots 3 and 4 and spots 5 and 6 predominate over the last two days.

Amphetamine excretion appears to be higher from an acid urine compared to an alkaline urine (30). Since the urine samples used in this experiment were slightly acidic (pH 6.5), it is possible that the higher excretion of amphetamine over the first two days could perhaps be partly due to these acidic conditions.

The high excretion of p-hydroxyamphetamine suggests that the compound is relatively stable in the cat.

The remaining metabolites appear to be retained by the body to be released slowly from their deposition sites.

Acid hydrolysis has shown that no conjugation of any metabolite was evident. This is expected in cats (54, 55). This also indicates that there is no apparent glucuronide formation as reported by some investigators (50).

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VI. APPENDICES

APPENDIX I

The following table gives a list of most of the amphetamine products available on the Canadian market.

COMMERCIAL AMPHETAMINE PREPARATIONS AVAILABLE IN CANADA

Trade Name	Dose	Dosage form	Manufacturer	Amphetamine Used
Ambar #1 and 2	1 before breakfast	Extentabs	A. H. Robins	Methamphetamine HCl
Amodex	1 cap. every morning 1 tab. two or three times daily	Capsules Tablets	E. L. Stickley	d-Amphetamine Sulfate
Amphetone	1 or 2 teaspoonfuls 3 times daily before meals	Liquid	Dymond	Methamphetamine HCl
Amphos	5-30 mg daily	Tablets	Pitman Moore	Amphetamine Phosphate
Anorex	1 cap. every morning 1 tab. three times daily	Capsules Tablets	Smallwood	d-Amphetamine Sulfate
Averdupo	1 tab. 2 or 3 times daily	Tablets	Dymond	Amphetamine Phosphate
Bamadex	1 cap. every morning 1 tab. three times daily	Capsules Tablets	Lederle	d-Amphetamine Sulfate

Trade Name	Dose	Dosage form	Manufacturer	Amphetamine Used
Barbidex #1 and #2	Take as directed	Capsules Tablets	Elliott-Marion	d-Amphetamine Sulfate
Benzedrine	As directed	Tablets	Smith Kline & French	dl-Amphetamine Sulfate
Biphetamine	1 every morning	Capsules	Strasenburgh	d-Amphetamine as cation exchange resin
Biphetamine T	1 every morning	Capsules	Strasenburgh	d and dl Amphetamine as cation exchange resin
Bitab #33	as directed	Tablets	Can. Pharm. Nordic	d-Amphetamine Sulfate
Butadex	1 two or three times daily	Tablets	Maney	d-Amphetamine Sulfate
Caltrol	1 three times daily	Tablets	Maney	dl-Amphetamine Sulfate
D-Ate	10-30 mg on arising	Capsules	Maney	d-amphetamine Sulfate
Daprisal	1 every 3 hours up to 6 daily	Tablets	Smith Kline & French	d-Amphetamine Sulfate

Trade Name	Dose	Dosage form	Manufacturer	Amphetamine Used
Daytone	1 tab. or 1 teaspoonful before meals	Tablets Elixir	Anca	Methamphetamine
Desanca	as directed	Tablets	Anca	Methamphetamine HCl
Desancabarb	1 or 2 teaspoonfuls before meals	Elixir	Anca	Methamphetamine HCl
Desbutal	as directed	Capsules Tablets	Abbott	Methamphetamine HCl
Desephrine	1-half hour before meals	Tablets	Can. Pharm. Nordic	Methamphetamine HCl
Desoxyn	as directed	Tablets	Abbott	Methamphetamine HCl
Dex	1 morning noon and night	Tablets	Stickley	dl-Amphetamine Sulfate
Dexabarb	1 or 2 before meals	Tablets	Gilbert	d-Amphetamine Sulfate
Dexamo #1, #2, #3	1 every morning	Capsules	Gilbert	d-Amphetamine Sulfate
Dexamobarb 15	1 every morning	Capsules	Dymond	d-Amphetamine Sulfate

Trade Name	Dose	Dosage form	Manufacturer	Amphetamine Used
Dexamyl #1 and #2	as directed	Capsules Tablets	Smith Kline & French	d-Amphetamine Sulfate
Dexavite	1 every morning	Capsules	Elliott Marion	d-Amphetamine Sulfate
Dexedrine	10-30 mg taken on arising	Tablets	Smith Kline & French	d-Amphetamine Sulfate
Dexellets	1 every morning	Capsules	Stickley	d-Amphetamine Sulfate
Dexobarb	1 - two or three times daily	Tablets	Ingram & Bell	Methamphetamine HCl
Dexobese A-B-C	1 before meals	Tablets	Bell-Craig	d-Amphetamine Sulfate
Dexobese V.M.	1 before breakfast	Capsules	Bell-Craig	d-Amphetamine Sulfate
Dexophen	as directed	Tablets	Corporation Pharmaceutique Francaise Ltd.	Methamphetamine HCl
Dexostan	10-30 mg taken on arising	Capsules	Stanley	d-Amphetamine Sulfate

Trade Name	Dose	Dosage form	Manufacturer	Amphetamine Used
Bexostan with Amobarbital	one on arising or as directed	Capsules	Stanley	d-Amphetamine Sulfate
Edrisal	2 every 3 hours. No more than 6-8 daily	Tablets	Smith Kline & French	dl-Amphetamine Sulfate
Eskatrol	1 every morning 1 three times daily	Capsules Tablets	Smith Kline & French	d-Amphetamine Sulfate
Ibadex	as directed	Tablets	Ingram & Bell	Methamphetamine HCl
Manedex #1, #2	1 every morning	Capsules	Maney	d-Amphetamine Sulfate
Menadis	as directed	Tablets	Eaton	Methamphetamine HCl
Methedrine	2.5-20 mg in the morning. 15-20 mg. IM or IV	Tablets Amps.	Burroughs Wellcome & Co.	Methamphetamine HCl
Neodrine	as directed	Tablets	Dymond	Methamphetamine HCl
Nobesine	1 every morning	Capsules	Nadeau	d-Amphetamine Sulfate

Trade Name	Dose	Dosage form	Manufacturer	Amphetamine Used
Nobesine-Hydro	1 every morning	Capsules	Nadeau	d-Amphetamine Sulfate
O. B. C.	as directed	Tablets	Stickley	dl-Amphetamine Sulfate
Obesamin	1 or 2 before meals	Tablets	Moore Thompson Clinger	dl-Amphetamine phosphate
Obesitabs	1/2 - 1 three times daily	Tablets	Moore Thompson Clinger	d-Amphetamine Phosphate
Obetrol	as directed	Tablets	Unik	Methamphetamine HCl Methamphetamine Saccharate dl-Amphetamine Sulfate d-Amphetamine Sulfate
Obocell	2-6 daily before meals	Tablets	Herdt & Charton	d-Amphetamine Phosphate
Probese A B C	as directed	Tablets	Bell Craig	dl-Amphetamine Sulfate
Probese T. D.	1 every morning	Capsules	Bell Craig	dl-Amphetamine Sulfate

Trade Name	Dose	Dosage form	Manufacturer	Amphetamine Used
Probese V.M.	1 every morning	Capsules	Bell Craig	dl-Amphetamine Sulfate
Pymadex	as directed	Tablets Vials	Stickley	d-Amphetamine Sulfate
Seco-Synatan	1 or 2 at 10 A.M.	Tablets	Herdt & Charton	d-Amphetamine Sulfate
Thyama	1 every morning	Capsules	Maney	dl-Amphetamine Sulfate
Thya Maney #1,#2,#3	as directed	Tablets	Maney	dl-Amphetamine Sulfate
Vitobese	1 before meals	Capsules	Can. Pharm.	d-Amphetamine Sulfate
Weightrol	1 before meals	Tablets	Smallwood	dl-Amphetamine Sulfate
Willedrine	as directed	Tablets	Will	Methamphetamine HCl

APPENDIX II

Table I

Quantitative distribution of ^{14}C-amphetamine metabolites obtained from the 24-hour urine sample

Cat	Activity dpm*	Spot #	Percentage of Total Activity
A	2,218	1	39.20
B	48,534	1	59.13
C	21,270	1	67.65
D	13,973	1	35.18
E	14,660	1	65.60
A	702	2	12.39
B	5,005	2	6.09
C	1,922	2	6.11
D	4,218	2	11.71
E	1,218	2	5.43
A	569	3 & 4(a)	10.04
B	1,952	3 & 4	2.37
C	944	3 & 4	3.00
D	1,923	3 & 4	4.85
E	978	3 & 4	4.37
A	2,174	5 & 6(b)	38.38
B	26,595	5 & 6	32.39
C	7,304	5 & 6	23.24
D	19,126	5 & 6	48.27
E	5,530	5 & 6	24.71

* Average of three replicates.

(a)(b) Spots 3 & 4, and spots 5 & 6 were counted together since it was not practical or statistically accurate to separate them individually.

Table II

Quantitative distribution of ^{14}C-amphetamine metabolites obtained from the 48-hour urine sample

Cat	Activity dpm*	Spot #	Percentage of Total Activity
A	20,008	1	67.49
B	7,439	1	41.51
C	3,791	1	49.80
D	2,156	1	49.19
E	5,035	1	17.03
A	1,148	2	3.87
B	487	2	2.72
C	660	2	8.68
D	665	2	16.13
E	1,274	2	4.30
A	1,209	3 & 4(a)	4.08
B	861	3 & 4	4.79
C	535	3 & 4	7.04
D	626	3 & 4	14.24
E	1,121	3 & 4	3.80
A	7,282	5 & 6(b)	24.56
B	9,135	5 & 6	50.97
C	2,626	5 & 6	34.48
D	896	5 & 6	20.43
E	22,130	5 & 6	74.86

*Average of three replicates.

(a)(b)Spots 3 & 4, and spots 5 & 6 were counted together since it was not practical or statistically accurate to separate them individually.

Table III

Quantitative distribution of ^{14}C-amphetamine metabolites obtained from the 72-hour urine sample

Cat	Activity dpm*	Spot #	Percentage of Total Activity
A	11,222	1	25.90
B	Not Seen	1	-
C	Not Seen	1	-
D	Not Seen	1	-
E	Not Seen	1	-
A	6,422	2	14.53
B	Not Seen	2	-
C	Not Seen	2	-
D	Not Seen	2	-
E	Not Seen	2	-
A	2,813	3 & 4(a)	6.49
B	457	3 & 4	26.80
C	352	3 & 4	35.71
D	491	3 & 4	39.33
E	365	3 & 4	34.74
A	22,974	5 & 6(b)	53.06
B	1,244	5 & 6	73.20
C	630	5 & 6	64.31
D	765	5 & 6	60.72
E	704	5 & 6	66.30

*Average of three replicates.

(a)(b)Spots 3 & 4 and spots 5 & 6 were counted together since it was not practical or statistically accurate to separate them individually.

Table IV

Quantitative distribution of ^{14}C-amphetamine metabolites obtained from the 96-hour urine sample

Cat	Activity dpm*	Spot #	Percentage of Total Activity
A	739	1	29.81
B	Not Seen	1	-
C	Not Seen	1	-
D	Not Seen	1	-
E	Not Seen	1	-
A	Not Seen	2	-
B	Not Seen	2	-
C	Not Seen	2	-
D	Not Seen	2	-
E	Not Seen	2	-
A	565	3 & 4(a)	22.93
B	552	3 & 4	52.17
C	554	3 & 4	57.60
D	320	3 & 4	45.91
E	459	3 & 4	35.03
A	1,168	5 & 6(b)	47.26
B	516	5 & 6	47.83
C	396	5 & 6	42.40
D	377	5 & 6	54.11
E	852	5 & 6	65.32

*Average of three replicates

(a)(b)Spots 3 & 4 and Spots 5 & 6 were counted together since it was not practical or statistically accurate to separate them individually.